CLAIMS

1. A method of introducing exogenous DNA into a preselected site of the genome of a primary or a secondary cell of vertebrate origin, comprising the steps of:
   a) transfecting the primary or the secondary cell with a DNA construct comprising exogenous DNA which includes DNA sequences homologous to genomic DNA sequences of the primary or secondary cell, thereby producing transfected primary or secondary cells and
   b) maintaining transfected primary or secondary cells under conditions appropriate for homologous recombination between DNA sequences in the DNA construct and genomic DNA to occur;
   thereby producing homologously recombinant primary or secondary cells.

2. The method of Claim 1 wherein the primary or secondary cell of vertebrate origin is selected from the group consisting of: fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, hepatocytes and precursors thereof.

3. The method of Claim 2 wherein the primary or secondary cell is of mammalian origin.

4. The method of Claim 3 wherein the primary or secondary cell is selected from the group consisting of: primary human cells, secondary human cells, primary mouse cells, secondary mouse cells, primary rabbit cells and secondary rabbit cells.

5. The method of Claim 4 wherein the exogenous DNA encodes a therapeutic product selected from the group consisting of: enzymes, cytokines, hormones, antigens, antibodies, clotting factors, regulatory proteins, ribozymes, transcription proteins, receptors, anti-sense nucleic acid sequences and novel proteins.

6. The method of Claim 4 wherein the exogenous DNA is itself a therapeutic product selected from the group consisting of DNA sequences sufficient for sequestration of a protein or nucleic acid in the cell, DNA sequences which bind to a cellular regulatory protein, DNA sequences which alter secondary or tertiary chromosomal structure and DNA sequences which are transcriptional regulatory elements.

7. The method of Claim 1 wherein the DNA construct of (a) additionally includes DNA encoding a selectable marker.

8. The method of Claim 7 wherein the primary or secondary cell is selected from the group consisting of: fibroblasts, keratinocytes, epithelial cells,

endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, hepatocytes and precursors thereof.

9. The method of Claim 1 further comprising culturing a homologously recombinant primary or secondary cell under conditions appropriate for propagating the homologously recombinant primary or secondary cell, thereby producing a clonal strain of homologously recombinant secondary cells.

10. A homologously recombinant primary or secondary cell produced by the method of Claim 1.

11. A clonal cell strain of homologously recombinant secondary cells produced by the method of Claim 9.

12. A method of targeting DNA sequences into genomic DNA of a primary or secondary cell of vertebrate origin, comprising the steps of:

a) providing a DNA construct comprising:
   1) exogenous DNA encoding a product to be expressed in primary or secondary cells of vertebrate origin;
   2) DNA sequences homologous with genomic DNA sequences in the primary or secondary cell of vertebrate origin; and
   3) DNA sequences encoding at least one selectable marker;

b) transfecting primary or secondary cells with the DNA construct provided in (a), thereby

producing primary or secondary cells containing the DNA construct provided in (a); and

c) maintaining primary or secondary cells produced in (b) under conditions appropriate for homologous recombination to occur between DNA sequences homologous with genomic DNA sequences and genomic DNA sequences,

thereby producing homologously recombinant primary or secondary cells of vertebrate origin having the DNA construct of (a) integrated into genomic DNA of the primary or secondary cells.

13. The method of Claim 12 wherein the primary or secondary cell is selected from the group consisting of: fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, hepatocytes and precursors thereof.

14. The method of Claim 13 wherein the primary or secondary cell is of mammalian origin.

15. The method of Claim 14 wherein the primary or secondary cell is selected from the group consisting of: primary human cells, secondary human cells, primary mouse cells, secondary mouse cells, primary rabbit cells and secondary rabbit cells.

16. The method of Claim 15 wherein the exogenous DNA encodes a therapeutic product select d from the group consisting of: enzymes, cytokines, hormones,

antigens, antibodies, clotting factors, regulatory proteins, ribozymes, transcription proteins, receptors, anti-sense nucleic acid sequences and novel proteins.

17. The method of Claim 16 wherein the exogenous DNA is itself a therapeutic product selected from the group consisting of DNA sequences sufficient for sequestration of a protein or nucleic acid in the cell, DNA sequences which bind to a cellular regulatory protein, DNA sequences which alter secondary or tertiary chromosomal structure and DNA sequences which are transcriptional regulatory elements.

18. A homologously recombinant primary or secondary cell produced by the method of Claim 12.

19. A homologously recombinant primary or secondary cell of vertebrate origin, having integrated into genomic DNA exogenous DNA.

20. A homologously recombinant primary or secondary cell of Claim 19 selected from the group consisting of: fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, hepatocytes and precursors thereof.

21. A homologously recombinant primary or secondary cell of Claim 20 which is of mammalian rigin.

22. A homologously recombinant primary or secondary cell of Claim 21 selected from the group consisting of: primary human cells, secondary human cells, primary mouse cells, secondary mouse cells, primary rabbit cells and secondary rabbit cells.

23. A homologously recombinant primary or secondary cell of Claim 22 wherein the exogenous DNA encodes a therapeutic product selected from the group consisting of: enzymes, cytokines, hormones, antigens, antibodies, clotting factors, regulatory proteins, ribozymes, transcription proteins, receptors, anti-sense nucleic acid sequences and novel proteins.

24. A homologously recombinant primary or secondary cell of Claim 22 wherein the exogenous DNA is itself a therapeutic product selected from the group consisting of DNA sequences sufficient for sequestration of a protein or nucleic acid in the cell, DNA sequences which bind to a cellular regulatory protein, DNA sequences which alter secondary or tertiary chromosomal structure and DNA sequences which are transcriptional regulatory elements.

25. A homologously recombinant primary or secondary cell of Claim 19 which additionally has integrated into genomic DNA DNA encoding a selectable marker.

26. A homologously recombinant primary or secondary cell of Claim 25 which is of mammalian origin.

27. A homologously recombinant primary or secondary cell of Claim 26 selected from the group consisting of: primary human cells, secondary human cells, primary mouse cells, secondary mouse cells, primary rabbit cells and secondary rabbit cells.

28. A homologously recombinant primary or secondary cell of Claim 27 wherein the exogenous DNA encodes a therapeutic product selected from the group consisting of: enzymes, cytokines, hormones, antigens, clotting factors, regulatory proteins, ribozymes, transcription proteins, receptors, an anti-sense nucleic acid sequence and novel proteins.

29. A homologously recombinant primary or secondary cell of Claim 27 wherein the exogenous DNA is itself a therapeutic product selected from the group consisting of DNA sequences sufficient for sequestration of a protein or nucleic acid in the cell, DNA sequences which bind to a cellular regulatory protein, DNA sequences which alter secondary or tertiary chromosomal structure and DNA sequences which are transcriptional regulatory elements.

30. A method of providing a therapeutic product in an effective amount to a mammal comprising the steps of:

a) providing a DNA construct comprising:
   1) exogenous DNA encoding a product to be expressed in primary or secondary cells of vertebrate origin;
   2) DNA sequences homologous with genomic DNA sequences in the primary or secondary cell of vertebrate origin; and
   3) DNA sequences encoding at least one selectable marker;

b) transfecting primary or secondary cells obtained from the mammal with the DNA construct provided in (a), thereby producing primary or secondary cells containing the DNA construct provided in (a);

c) maintaining primary or secondary cells produced in (b) under conditions appropriate for homologous recombination to occur between DNA sequences homologous with genomic DNA sequences and genomic DNA sequences,

thereby producing homologously recombinant primary or secondary cells of vertebrate origin having the DNA construct of (a) integrated into genomic DNA of the primary or secondary cells; and

d) introducing homologously recombinant primary or secondary cells produced in (c) into the mammal in sufficient number to produce an effective amount of the therapeutic product in the mammal.

31. The method of Claim 30 wherein the primary or secondary cell is selected from the group consisting

of: fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, hepatocytes and precursors thereof.

32. The method of Claim 31 wherein the primary or secondary cell is selected from the group consisting of: primary human cells, secondary human cells, primary mouse cells, secondary mouse cells, primary rabbit cells and secondary rabbit cells.

33. The method of Claim 32 wherein the exogenous DNA encodes a therapeutic product selected from the group consisting of: enzymes, cytokines, hormones, antigens, antibodies, clotting factors, regulatory proteins, ribozymes, transcription proteins, receptors, anti-sense nucleic acid sequences and novel proteins.

34. The method of Claim 32 wherein the exogenous DNA is itself a therapeutic product selected from the group consisting of DNA sequences sufficient for sequestration of a protein or nucleic acid in the cell, DNA sequences which bind to a cellular regulatory protein, DNA sequences which alter secondary or tertiary chromosomal structure and DNA sequences which are transcriptional regulatory elements.

35. A method of targeting exogenous DNA into a preselected site in genomic DNA of a primary or

secondary cell of vertebrate origin, comprising the steps of:

a) providing a DNA construct comprising:

1) exogenous DNA selected from the group consisting of:

a) DNA sequences which repair, alter, delete or replace a resident gene in the primary or secondary cell;

b) DNA sequences encoding a product not normally expressed in the primary or secondary cells or not expressed in significant levels in the primary or secondary cells as obtained;

c) DNA sequences which repair, alter, delete or replace a regulatory sequence present in the primary or secondary cells;

d) DNA sequences which encode a regulatory sequence not normally functionally linked to a gene to be expressed in the primary or secondary cells as obtained; and

e) DNA sequences which inactivate or remove a gene or gene portion in the primary or secondary cells;

2) DNA sequences homologous with genomic DNA sequences in the primary or secondary cells; and

3) DNA sequences encoding at least one selectable marker;

b) transfecting primary or secondary cells with the DNA construct provided in (a), thereby producing primary or secondary cells containing the DNA construct provided in (a); and

c) maintaining primary or secondary cells produced in (b) under conditions appropriate for homologous recombination to occur between DNA sequences homologous with genomic DNA sequences and genomic DNA sequences,

thereby producing primary or secondary cells of vertebrate origin having the DNA construct of (a) integrated into genomic DNA of the primary or secondary cells.

36. A method of increasing the efficiency of homologous recombination between a) genomic DNA sequences of a primary or a secondary cell of vertebrate origin and b) DNA sequences, present in a DNA construct, which are homologous with genomic DNA sequences of the primary or the secondary cell, comprising introducing into the primary or the secondary cell a linear DNA construct having a single stranded overhang at each end.

37. A method of turning on expression of a gene to be expressed which is present in a cell but is not expressed in the cell as obtained or is not expressed at significant levels in the cell as obtained, comprising introducing into the cell a DNA construct comprising a regulatory region under conditions appropriate for homologous recombination,

whereby the regulatory region is inserted into or replaces all or a portion of the regulatory region of the gene to be expressed, and is functionally linked to the gene to be expressed, thereby producing homologously recombinant cells which express the gene.

38. The method of Claim 37 wherein the gene is selected from the group consisting of: the human erythropoietin, growth hormone, and insulin genes.

39. The method of Claim 37 wherein the cell is a primary or secondary cell of vertebrate origin selected from the group consisting of: fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, hepatocytes and precursors thereof.

40. The method of Claim 39 wherein the primary or secondary cell is of mammalian origin.

41. The method of Claim 40 wherein the primary or secondary cell is selected from the group consisting of: primary human cells, secondary human cells, primary mouse cells, secondary mouse cells, primary rabbit cells and secondary rabbit cells.

42. The method of Claim 37 wherein the gene to be expressed encodes a product selected from the group consisting of: enzymes, cytokines, hormones,

antigens, antibodies, clotting factors, regulatory proteins, transcription proteins and receptors.

43. A method of targeting DNA sequences into genomic DNA of a primary or secondary cell of vertebrate origin, comprising the steps of:

a) providing a DNA construct comprising:

1) exogenous DNA encoding a product to be expressed in primary or secondary cells of vertebrate origin;

2) DNA sequences homologous with genomic DNA sequences in the primary or secondary cell of vertebrate origin; and

3) DNA sequences encoding at least one selectable marker;

b) transfecting primary or secondary cells with the DNA construct provided in (a), thereby producing primary or secondary cells containing the DNA construct provided in (a); and

c) maintaining primary or secondary cells produced in (b) under conditions appropriate for homologous recombination to occur between DNA sequences homologous with genomic DNA sequences and genomic DNA sequences, thereby producing homologously recombinant primary or secondary cells of vertebrate origin having the DNA construct of (a) integrated into genomic DNA of the primary or secondary cells;

d) exposing the homologously recombinant primary and secondary cells produced in (c) to a selectiv agent which sel cts f r the

selectable marker present in the targeting DNA construct, whereby cells that have not stably integrated the selectable marker are killed and cells that have stably integrated the selectable marker can survive and form colonies; and

e) screening colonies produced in (d) to identify homologously recombinant primary or secondary cell strains.

44. The method of Claim 43 wherein the positive selective marker is neo and the selective agent is G418.

45. A method of targeting DNA sequences into genomic DNA of a primary or secondary cell of vertebrate origin, comprising the steps of:

a) providing a DNA construct comprising:

1) exogenous DNA encoding a product to be expressed in primary or secondary cells of vertebrate origin;

2) DNA sequences homologous with genomic DNA sequences in the primary or secondary cell of vertebrate origin; and

3) DNA sequences encoding at least one each positive and negative selection markers, in such a configuration that homologous recombination between the targeting sequences and homologous sequences in the host cell genome results in the targeted integration of the positive s lection

marker, while the negative selection marker is not integrated;

b) transfecting primary or secondary cells with the DNA construct provided in (a), thereby producing primary or secondary cells containing the DNA construct provided in (a); and

c) maintaining primary or secondary cells produced in (b) under conditions appropriate for homologous recombination to occur between DNA sequences homologous with genomic DNA sequences and genomic DNA sequences, thereby producing homologously recombinant primary or secondary cells of vertebrate origin having the DNA construct of (a) integrated into genomic DNA of the primary or secondary cells;

d) exposing primary and secondary cells produced in (c) to a selective agent which selects for the positive selection marker present in the targeting DNA construct, whereby cells that have not stably integrated the positive selection marker are killed and cells that have stably integrated the positive selection marker can survive and form colonies; and

e) additionally exposing the cells produced in (c) and (d) to an agent which selects against the negative selection marker present in the targeting DNA construct, whereby cells that have stably integrated the negative selection marker are killed, with the result of steps (d) and (e) being such that homologously recombinant cells are selected.

46. The method of Claim 45 wherein the primary or secondary cell is selected from the group consisting of: fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, hepatocytes and precursors thereof.

47. The method of Claim 46 wherein the primary or secondary cell is of mammalian origin.

48. The method of Claim 47 wherein the primary or secondary cell is selected from the group consisting of: primary human cells, secondary human cells, primary mouse cells, secondary mouse cells, primary rabbit cells and secondary rabbit cells.

49. The method of Claim 48 wherein the exogenous DNA encodes a therapeutic product selected from the group consisting of: enzymes, cytokines, hormones, antigens, antibodies, clotting factors, regulatory proteins, ribozymes, transcription proteins, receptors, anti-sense nucleic acid sequences and novel proteins.

50. The method of Claim 49 wherein the exogenous DNA is itself a therapeutic product selected from the group consisting of DNA sequences sufficient for sequestration of a protein or nucleic acid in the cell, DNA sequences which bind to a cellular regulatory protein, DNA sequences which alter secondary or tertiary chromosomal structure and DNA

sequences which are transcriptional regulatory elements.

51. A homologously recombinant primary or secondary cell produced by the method of Claim 45.

52. A clonal cell strain of homologously recombinant secondary cells produced by the method of Claim 45.

53. The method of Claim 45 in which the positive selective marker is neo and the selective agent is G418.

54. The method of Claim 45 in which the negative selection marker is gpt and the negative selective agent is 6-thioxanthine.

55. The method of Claim 37 wherein the cell as obtained is an immortalized cell.

56. The method of Claim 45 in which the negative selection marker is the HSV-TK gene and the negative selective agent is gancyclovir.

57. The method of Claim 45 in which two negative selection markers are used, where one negative selection marker is gpt and one negative selection marker is the HSV-TK gene.

58. A homologously recombinant primary or secondary cell produced by the method of Claim 37.

59. A clonal cell strain of homologously recombinant secondary cells produced by the method of Claim 37.

60. The method of Claim 37 in which the DNA construct additionally comprises a positive selection marker.

61. The method of Claim 37 in which the DNA construct additionally comprises at least one positive selection marker and at least one negative selection marker.

62. The method of Claim 60 in which the positive selection marker is neo and the positive selection agent is G418.

63. The method of Claim 61 in which the positive selection marker is neo and the positive selection agent is G418, and the negative selection marker is gpt and the negative selection agent is 6-thioxanthine.

64. The method of Claim 61 in which the positive selection marker is neo and the positive selection agent is G418, and the negative selection marker is the HSV-TK gene and the negative selection agent is gancyclovir.

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